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AN EPIDEMIC AMONG ENGLISH SPARROWS DUE TO BACILLUS CLOACAE.*

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THE frequent outbreaks of sickness among farm poultry, together with the occasional epidemics which occur in the aviaries of bird-fanciers, have led to a more or less careful study of the cause of these diseases.

In 1893 Klein[1] examined a number of pheasants which succumbed during the rearing at Hawarden, England, and isolated a bacillus which he describes as resembling very closely *B. coli*. The only differences noted between the organism found in the pheasants and *B. coli* were that the former was more actively motile, shorter, and did not coagulate milk.[2] Two young pheasants, four young chickens, two pigeons, two half-grown chickens, two rabbits, and two guinea-pigs were inoculated with pure cultures. The two pheasants were found dead the next day. All the other animals survived. Sanfelice[3] obtained an organism from infected pigeons which he thought identical with *B. coli*. The bacillus was obtained from the liver, spleen, and heart's blood. Pigeons inoculated subcutaneously with 1 to ½ c.c. of a broth culture of this organism developed abscesses at the point of inoculation, but pigeons, inoculated intraperitoneally with even smaller doses, died in 24 hours. Kern[4] studied an epidemic which occurred among canaries in Budapest. Two new birds were bought and introduced into the aviary of the veterinary academy and in 22 days all but three of the birds in the aviary had died one after the other. Birds were found on the floor of the cages doubled up or on their backs. A bacillus was isolated in pure culture from the heart's blood, and other birds were inoculated. Some of these died in from four to six days. Pigeons and chickens were inoculated with negative results. Some positve and some negative results were obtained with sparrows. The specific bacteria were recovered from birds which died from the injection experiments. Tartakowsky[5] described an epidemic which occurred among the small birds of St. Petersburg and isolated an organism to which he gave the name of "*Bacillus laxiacidae.*" He called the disease seticemic enteritis of sparrows. The bacillus was said to resemble *B. coli* in its characteristics; but it had some resemblance also to *B. psittacosis* so that this author was undecided as to which class it belonged. Miessner[6] and Schern, in November, 1906, investigated the cause of death of a number of canaries and isolated, from the spleen and liver of these birds, an organism which they consider to be different from any previously described. The organism is described as a plump, non-motile bacillus rounded on the ends. It stains fairly well with ordinary stains but is negative to Gram. It grows on all culture media, but better on glycerin agar or in glycerin broth. These

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[1] *Jour. Path. and Bact.*, 1894, 11, p. 214.

[2] Klein does not state the time for which the milk was incubated.

[3] *Ztschr. f. Hyg.*, 1895, 20, p. 23.

[4] *Deut. Ztschr. f. Tiermed.*, 1896, 22, p. 171.

[5] *Centralbl. f. Bakt.*, 1899, 25, p. 89.

[6] *Arch. f. wissensch. u. prakt. Thierh.*, 1908, 34, p. 133.

authors state that the organism was obtained in every case from the liver and spleen, but never from the blood of the birds. They decided that the organism so discovered was a "Bacterium sui generis," and burdened it with the name *B. canariensis necrophorus*. Zwick[1] studied the cause of death of a number of canaries which succumbed during an epidemic which occurred in the cages of one of the bird-fanciers of Stuttgart, Germany. He isolated an organism from the blood of the birds which he described as a small, non-motile bacillus, rounded on the ends and negative to Gram. Birds which were inoculated with this organism are said to have died in from 24 to 48 hours after the injection. Rettger and Harvey[2] have recently isolated an organism from young chickens which had died from so-called "white diarrhea." The organism is described as a long, slender, non-motile bacillus, slightly rounded on the ends, which, in its microscopic appearance, resembles *B. typhosus*. On agar slants, according to these authors, growth is visible in 24 hours, but it spreads little and remains delicate even after prolonged incubation. On potato, an almost invisible streak is produced along the whole line of inoculation. In litmus milk, little or no change occurs within the first 48 hours. Thereafter, the milk becomes slightly acid; but it is not coagulated. Gelatin is not liquefied. Gas is produced in dextrose fermentation tubes, but maltose, lactose, saccharose, inulin, and dextrin, according to these authors, are not fermented.

The bacterial infection of birds, considered in this paper, was observed in 23 English sparrows, *Passer domesticus*, which were caught in a figure-four trap and transferred to large cages without any unnecessary handling. All the birds, which were captured for another purpose, were apparently healthy when placed in the cages. The cages were carefully cleaned and disinfected before the birds were placed in them and again after the death of any of the birds, before any more were introduced. Every care was taken of the birds, but they continued to die, in from four days to two weeks after being brought into captivity.

The symptoms in all cases were similar. When first put into the cage, the birds were extremely active. In two or three days, they became quiet, lost their appetite, stood on the floor with feathers ruffled up and eyes closed. They became extremely weak. Toward the end, if placed on their backs, they were too weak to right themselves. In the last stages of their sickness, they stood on the floor of the cage doubled up and appearing to suffer pain. Some died doubled up; others were found on their backs dead. In several cases the birds, apparently recovered from the first attack, began to hop around and eat again, but a second attack soon followed, and, in every case, this proved fatal.

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[1] *Ztschr. f. Hyg. d. Haustiere*, 1908, 4, p. 33.

[2] *Jour. Med. Res.*, 1908, 18, p. 277.

Autopsy showed the spleen swollen. In several cases, when the blood vessels were cut, gas bubbled out. In four or five cases, this happened when the vessels were cut before the death of the animals. Drops of blood taken from a small vein under the wing, one to two hours before death, revealed under the hanging drops small, actively motile bacilli which appeared to be in pure culture. Smears were made from blood taken from the vein of the wing and stained. Microscopical examination of the stained smears showed the absence of all other organisms.

Smears made from the heart's blood on slant agar and in broth gave pure cultures. Out of 23 birds which died, pure cultures of a specific bacillus were obtained from 15 and the same organism was obtained by plating out from a mixture of bacteria in the other cases. The bodies of the latter had stood so long before the examination of them that a post-mortem invasion of the body had taken place. In four cases, cultures made from the blood taken from a branch of the humeral vein and obtained before the death of the animal gave pure cultures of the organism.

Description of the bacillus.—The organism is a small, very actively motile bacillus (0.7 to 1.5 μ long), rounded on the ends. It grows well at 37° C., and at room temperature, both under aerobic and anaerobic conditions, the latter tests being made according to Buchner's method. The bacillus stains well with ordinary stains, but is Gram-negative. No capsules or involution forms were noted.

Cultural characteristics.—Smears made on agar slants gave an abundant spreading, raised, translucent, glistening, smooth growth. Older cultures become slightly opalescent.

On potato, an abundant, speading, raised, slimy growth is produced. The medium is darkened. Gelatin is liquefied in from 9 to 12 days when kept at 22° C. Broth becomes uniformly cloudy in 18 hours. Litmus milk is rendered slightly acid in 24 hours. The acidity increases slowly until the milk is coagulated, which takes from six to eight days. The coagulation takes place very slowly, the milk becoming thicker and thicker until a solid mass is formed. The milk has a slight aromatic odor.

The production of gas in glucose agar is profuse. In almost every case, the agar was found split up and the plugs blown out of the tubes in 24 hours; rarely did it take as long as 48 hours for this to take place.

In lactose agar, the growth follows the needle-track; but the production of gas is variable. In some cases, gas has been observed, but in most cases, gas was not produced when sugar-free beef extract had been used in making the agar.

On agar plates, the bacilli appear as small white pin-head colonies.

In 24 hours, the reaction in glucose-litmus-agar plates is acid, the plates being completely red: but, in from two to three days, the plates again become alkaline. The reaction in lactose-litmus plates remains alkaline.

In dextrose fermentation tubes, 100 per cent of gas is invariably produced in 48 hours The gas ratio, H:CO_2, is 1:2. Gas production in lactose fermentation tubes varies. If gas is produced at all, it is produced very slowly and in small quantities. In a number of cases, a small amount of gas has been noted in from 48 to 72 hours. Galactose, maltose, saccharose, mannite, and levulose are all readily fermented.

In order to trace more accurately the change of the reaction in dextrose-litmus agar, 100 c.c. of dextrose broth were placed in a small flask and sterilized. After sterilization, the broth gave a neutral reaction. This broth was inoculated with the organism, incubated at 37°, and titrated every 12 hours for four days.

The acidity of dextrose broth inoculated with bird organism was as follows:

Hours:	0	12	24	36	48	60	72	84	96
Per cent:	0	1.6	2	2.2	2.4	1.8	1.2	0.8	0.15

These results show an increase of acid up to 48 hours; but a gradual decrease thereafter until the fourth day when the reaction becomes almost neutral to phenolphthalein or alkaline to litmus. This phenomenon is possibly due to the fact that the organism attacks the carbohydrates much more readily than the proteins. When the carbohydrates are used up, the proteins are then attacked and NH_3 is split off. This neutralizes the acid formed from the fermentation of the sugars. Probably the two reactions are going on simultaneously, but the formation of acid is so much faster than the liberation of NH_3 during the first 48 hours that the latter reaction is masked.

Pathogenicity.—One c.c. of 24-hour broth culture of the bacillus was injected into the pectoral muscle of a pigeon. The bird showed effects in about 24 hours. It refused to eat, stood on the floor of the cage, and revealed a number of symptoms noted in the sparrows. It lost weight continually for about five days. It then gradually recovered and again became active. During its illness, the excretions were very watery and consisted mostly of mucus. A small loopful of this mucus-like substance was transferred to a melted glucose-agar tube: this was poured into a sterile petri dish and incubated at 37° for 24 hours. From the large number of small white colonies which appeared on the plate, bacilli which gave all the cultural reactions of the bacillus found in the sparrows were fished out. A second pigeon was inoculated with a like amount of the same organisms. The effects were the same in both cases.

Since the excretions of the pigeons were not examined for the bacillus before inoculation, it is not certain that it is not present normally in the intestinal tract of the birds. The great number found on the plates would seem to indicate this. The diarrheal discharge may have been induced by some toxic substance produced by the injected bacteria The excretions became normal again when the symptoms disappeared. Three sparrows were inoculated and these died in from two to three days after inoculation. Since all the sparrows which had died previously succumbed in from two days to two weeks after being placed in the cages, it is difficult to conclude that the death of those inoculated was due to the injection.

Three guinea-pigs were inoculated subcutaneously with 2 c.c. of a suspension of a 24-hour agar culture of the bacillus in physiological-salt solution. In two days, one developed an induration at the point of inoculation. The other two either gave negative results or the effects were too slight to be noticed.[1] The bacillus was recovered from the serous exudate after four days and cultivated on nutrient agar. A second guinea-pig was inoculated subcutaneously with 2 c.c. of a suspension of a 24-hour agar culture of the recovered bacillus. In two days it developed a large induration which became soft and broke on the third day. The bacillus recovered from the second animal was passed through a third guinea-pig and the bacillus obtained from an induration produced in this animal was tested on all the ordinary media and it agreed with the original bacillus. This bacillus was used in the experiments that follow:

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[1] Six animals had been inoculated with the bacillus before these but all gave negative results.

Two guinea-pigs, weighing 290 gm. each, were inoculated intraperitoneally with 1 c.c. of a suspension of a 24-hour agar culture. Both died in less than 18 hours. Animals inoculated with smaller doses lost in weight for a time, but recovered. Animals inoculated subcutaneously with 2 c.c. of a suspension of the recovered bacillus all developed indurations at point of inoculation, in two cases of which the bacillus was again recovered. No attempt was made to obtain the organism in the other two cases. In all fatal cases, the bacillus was recovered from the purulent exudate in the peritoneal cavity, and in two cases it was recovered from exudates in the pleural cavity and from the heart.

Guinea-pigs which were inoculated both subcutaneously and intraperitoneally with the original organism all recovered.

A rabbit, weighing 1,590 gm., was inoculated intraperitoneally with 2 c.c. of a suspension of a 24-hour agar culture from an induration. The next morning it was found doubled up in the corner of the cage. When agitated, it refused to move and did not eat when fed. It seemed to be extremely weak. In 48 hours it had lost 200 gm. in weight. The loss in weight continued until the fourth day when the animal moved about a little, but still seemed weak and stupid. On the fifth day, it ate when fed and began to move about more actively. From this time on, a gradual recovery was noted.

All the organisms recovered from the dead animals and those recovered from indurations were tested on all the ordinary culture media. The reactions varied little from those given by the original culture.

From a careful study of the morphology, cultural reactions, and even the pathogenicity of this bacillus, the resemblance to bacilli of the proteus group was evident. In order to study this resemblance more carefully and also to compare its reactions with those of other intestinal organisms, comparative tests were made with stock cultures of *B. proteus vulgaris*, *B. zenkeri*, *B. proteus mirabilis*, *B. cloacae*, *B. paratyphosus*, *B. enteridis*, *B. coli*, and the *B. cholerae suis*. The bacillus under study resembled in both morphological and cultural characteristics the stock culture of *B. cloacae* (Jordan) except that it is more actively motile. It differs from *B. coli* in being more actively motile and in producing more gas in 24 hours than is usually produced in the same length of time by *B. coli* and in the liquefaction of gelatin. The time necessary for the coagulation of milk is also much longer for the bacillus isolated from birds than for *B. coli* and the gas ratio is reversed.

Agglutination.—Two healthy rabbits were used in the immunization experiment. These animals were immunized by means of suspensions of the bacillus, isolated from the sparrows, in physiological-salt solution. Inoculations were made subcutaneously every day for eight successive days, then every other day for three weeks. The initial dose was 1 c.c. of a suspension of the bacilli in sterile physiological-salt solution which had been heated at 65° C. for five minutes. This was followed on the second day with 1 c.c. of an unheated suspension of the same organism. The doses were gradually increased until 4 c.c. of an unheated suspension of the bacilli were injected at one time. The animals were anaesthetized and 5 c.c. of blood were taken from the heart in each case. This was centrifugalized and the serum was used in the agglutinating experiments.

The bacillus found in the birds was agglutinated by the immunized rabbit serum in dilutions as high as 1: 50,000 in four hours. The stock culture of *B. cloacae* was agglutinated completely in 20 hours in dilutions of 1: 100 and 1: 200 but less completely in dilutions of 1: 500 and 1: 1,000, while *B. coli* and *B. vulgaris* were both completely agglutinated in dilutions as high as 1: 50 but less completely in dilutions of 1: 100 and 1: 200.

If the cultural and morphological characteristics of the bacillus isolated from sparrows in this laboratory be compared with the reactions described by a number of previous observers as characteristic of organisms isolated from the blood and organs of birds during an epidemic among these animals, a striking resemblance will be noted. In some cases, these bacteria were described as *B. coli* or coli-like organisms; in other cases, where the differences were more marked or the study made more carefully, new names were given. A careful examination of the cultural reactions of the coli-like organisms as described by some observers shows many variations from the *B. coli* type. If, however, these reactions be compared with the cultural characteristics of *B. cloacae* the resemblance is so marked that one is inclined to believe that these observers were studying a bacillus of the proteus group and not a variety of *B. coli.* Since I have demonstrated that the bacillus isolated from the birds, which had died from an infection produced by it, can be rendered pathogenic, not only for pigeons, but for rabbits and guinea-pigs as well, and that this organism seems to be in all respects identical with that first isolated by Jordan from sewage and called by him *B. cloacae*, one is inclined to think, at least, that *B. coli* has been accused of producing many diseases for which *B. cloacae* is responsible. Since both organisms are found in the intestines of animals and hence in sewage, together with the fact that they resemble each other very closely in cultural reactions, it is probable that the differences between these two bacilli may have been overlooked by some observers.

SUMMARY AND CONCLUSION.

1. A bacillus isolated from the blood of sparrows during an epidemic among these birds was the probable cause of their death, since it was obtained in pure culture from all that died and similar symptons were produced in pigeons inoculated with it.

2. By passing this bacillus through animals, its virulence was increased so that it became pathogenic for guinea-pigs and rabbits.

3. So far as can be determined from the description given by other observers this bacillus resembles very closely certain bacteria isolated by them in bird epidemics and called by them *B. coli* or coli-like organisms.

4. The bacillus found in sparrows gives all the cultural characteristics of *B. cloacae* (Jordan) a member of the proteus group.

5. The serum of rabbits injected with the bacillus found in the sparrows will agglutinate the homologous organism in dilutions as high as 1:50,000.

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